Journal of Medical Virology 53:25–30 (1997)

Follow-Up of Four HIV-Infected Individuals After Administration of Hepatitis C Virus and GBV-C/Hepatitis G Virus Contaminated Intravenous Immunoglobulin: Evidence for HCV But Not for GBV-C/HGV Transmission

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In 1994, hepatitis C virus (HCV) infection was transmitted to four HIV seropositive patients attending the Department of Angiology, University Clinics, Frankfurt am Main, by the administration of Gammagard®. The patients were suffering from thrombocytopenia and received betweeen 20 and 30 g of the contaminated lot 93F21AB11. GBV-C/HGV RNA could be amplified from the Gammagard® lot 93F21AB11 using 5′NCR and NS5 primer pairs. All the four patients were negative in the GBV-C/HGV RT-PCR prior to therapy and until the end of the follow-up period. GBV-C/HGV IgG antibodies to the putative envelope (E2) were detected using the E2 HGV-env kit (Boehringer-Mannheim, Germany) in Gammagard® lot 93F21AB11 and in one patient before donation of immunoglobulin. Anti-E2 seroconversion was observed in one recipient, the other two patients remained anti-E2 seronegative until the end of the observation period. It is concluded that there is no direct evidence for transmission of GBV-C/HGV by contaminated intravenous immunoglobulin since GBV-C/HGV RNA was not detected in the recipients up to 1 year after administration. ***J. Med. Virol. 53:25–30, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: acute HCV infection; GBV-C/HGV; HIV; PCR; anti-E2 antibody; intravenous immunoglobulin

INTRODUCTION

Human immunglobulin G preparations suitable for intravenous use (IVIG) are used increasingly for the prophylaxis of infections in patients with primary and secondary immunodeficiency and for the treatment of a wide variety of immunologically mediated disorders [Yap et al., 1994]. Gammaglobulin preparations may still carry a limited infection risk due to residual viral contamination although several methods to abolish or reduce infectivity have been developed [for review see Rabenau, 1995]. In recent years, several outbreaks of blood-borne HIV, HAV, HBV, HCV, and parvovirus B19 infections have been reported. In February 1994, Gammagard®, an IVIG preparation, manufactured by Baxter Healthcare Corporation Hyland Division (Glendale, USA), was withdrawn since several cases of hepatitis C infection had been reported. Meanwhile, approximatively 200 cases have been observed worldwide [Yu et al, 1995].

Since the newly discovered flavivirus GBV-C/HGV is a transfusion-transmissible agent, which is prevalent in up to 1% of volunteer blood donors and a high proportion of plasma pools and several Gammagard® batches yield GBV-C/HGV RNA, there may be also a potential risk for GBV-C/HGV transmission by IVIG [Jarvis et al., 1996; Linnen et al., 1996; Nübling et al., 1995; Simons et al., 1995].

The present report describes four cases of acute HCV infection in HIV seropositive individuals suffering from severe thrombocytopenia who had been treated with the HCV contaminated Gammagard® lot 93F21AB11 in January and February of 1994. These patients had been followed-up prospectively with regard to HCV RNA load, anti-HCV immune response, and biochemical markers of liver injury. In order to assess the potential risk of GBV-C/HGV transmission GBV-C/HGV RNA and antibody determination were carried out ret-

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Accepted 9 April 1997

TABLE I. Primer and Probe Sequences for the GBV-C/HGV-PCR

Primer and probes	Sequence	Position in the GBV-C/HGV-genome
5′-NCR region		
Primer 1	5’-Cgg-CCA-AAA-ggT-ggT-ggA-TG-3’	101–120
Primer 2	5’-CgA-CgA-gCC-TgA-CgT-Cgg-g-3’	285–267
Capture probe	5’-biotin-ggT-AgC-CAC-TAT-Agg-Tgg-g-3’	161–179
NS5a region		
Primer 1	5’-CTC-TTT-gTg-gTA-gTA-gCC-gAg-AgA-T-3’	77–101
Primer 2	5’-CgA-ATg-AgT-CAg-Agg-ACg-ggg-TAT-3’	211–188
Capture probe	5′-biotin-gTT-ACT-gAg-AgC-AgC-TCA-gAT-3′	152–172

rospectively in the Gammagard® lot and plasma and serum samples from the four HCV infected patients.

PATIENTS AND METHODS

Four patients (1 male, 3 female) with HIV-associated severe hypogammaglogulinemia were treated with intravenous immunoglobulin (Gammagard®, Baxter Healthcare Corporation, Hyland Division, Glendale, USA) for several months in the Department of Angiology outpatient clinic, University Clinics Frankfurt/Main. The HCV contaminated lot 93F21AB11 was administered in January and February of 1994. Two patients received the contaminated lot two times (first: 15 g = 300 ml, second: 15 g = 300 ml 1 month later). The other two patients received the contaminated lot only once (20 g = 400 ml). The recipients were HCV seronegative and HCV RT-PCR negative prior to Gammagard® therapy.

HCV Antibody Tests

Anti-HCV EIA second generation and HCV Matrix (Abbott Laboratories, Delkenheim, Germany) were carried out and interpreted according to the manufacturer‘s recommendations.

Detection of HCV-RNA

For qualitative HCV RT PCR, 140 μl serum were extracted with Qiagen HCV Kit (Qiagen, Hilden, Germany) according to the manufacturer‘s instructions. After reverse transcription using a RNA PCR kit (Perkin Elmer, Langen, Germany) nested-PCR (35 cycles) was performed with primers of the 5′-noncoding region of HCV as previously described [Berger et al., 1996]. Detection was done on 20% polyacrylamide gels after silver staining (PhastSystem, Pharmacia, Freiburg, Germany).

Quantitative HCV RT-PCR (HCV Monitor Test, Hoffmann-La Roche, Basel, Switzerland) was performed according the manufacturer‘s recommendations. This assay includes a quantitation standard (QS) that is co-amplified with the target, to monitor the efficiency of the amplification reaction. After amplification, the products were denatured, transfered to a microwell detection plate, and diluted serially twice and hybridized to HCV or QS specific probes in separate wells. The highest dilution that gave an extinction (A_{450}) between 0.5 and 2.0 on the HCV or QS-specific wells were selected to calculate the HCV-RNA copy number.

HCV genotyping was carried out using a type specific detection system of the PCR amplified 5′-noncoding region of HCV (Inno-LiPA, Innogenetics, Zwijnaarde, Belgium) according to the manufacturer’s instructions. Briefly, the highly conserved 5′-noncoding region of the HCV genome was amplified by HCV RT PCR using universal biotinylated HCV primers. Specific oligonucleotide-probes immobilized as parallel lines on membrane strips were hybridized with the biotinylated amplification products. The reactivity of the amplified fragment with one or more lines on the strip allowed the recognition of HCV genotypes.

GBV-C/HGV-RT-PCR

RNA was isolated from 140 μl serum by using the Qiamp HCV Kit (Qiagen, Hilden, Germany) according to the manufacturer‘s recommendations. cDNA synthesis was carried out in a 20 μl reaction volume at 42°C for 15 min using Random Hexamers (50 μM, Perkin Elmer, Langen, Germany), 25 U/μl MuLV reverse transcriptase (Perkin Elmer, Langen), 20 U/μl RNase inhibitor (Perkin Elmer), and 1 mM (each) deoxnucleotide (Pharmacia, Freiburg, Germany). The reaction was stopped by heating to 99°C and cooling to 4°C. Primer sequences were derived from the 5′-nontranslated (5′NCR) and the NS5-region of the GBV-C/HGV genome (Table I). For the detection with the Enzymun assay, 1 μl digoxigenin-dUTP was employed. Amplification of cDNA was carried out in a final volume of 50 μl of each 0,25 μM primer probe (Hepatitis G Virus Primer and Capture Probe Set, Boehringer Mannheim, Germany), PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$), and 2 U Taq DNA Polymerase (Boehringer-Mannheim, Germany). Amplification involved 35 cycles (GeneAmp PCR system 9600, Perkin Elmer) with denaturation at 95°C (30 sec), annealing at 50°C (30 sec), and extension at 72°C (30 sec). After the final cycle, the tubes were incubated for further 5 min at 72°C. Each test series included positive and negative controls.

Amplification products were detected by using the Enzymun-test DNA detection kit (Boehringer-Mannheim, Germany). Denaturation was carried out in sample tubes containing 40 μl of the PCR mixture

and 360 μl of 50 mM NaOH. All subsequent steps were automatically done by the Boehringer ES 300 analyzer. Briefly, 100 μl of denaturated amplicons were transferred with 400 μl of the virus-specific 5′-biotin-labelled capture probe (14 ng/μl diluted in hybridization buffer) into a streptavidin-coated tube, and the mixture was left for 120 min at 37°C. After extensive washing the peroxidase-labelled anti-digoxigenin polyclonal antibody was added, incubated for 30 min at 37°C and after washing, the enzyme substrate (1,9 mM 2,2′-aminobis (3-ethylbenzthiazoline sulfonic acid), 100 mM phosphate citrate buffer (pH 4.4, with 3.2 mM H_20_2) was added. The colour reaction was allowed to develop for 30 min at 37°C, and ODs were measured at 422 nm. The cut-off was defined as two times the OD obtained with negative controls.

RESULTS

All four patients were HCV seronegative and HCV RT-PCR negative prior to Gammagard® therapy and became HCV infected as documented by HCV RNA detection in serum samples obtained thereafter. The Gammagard® lot 93F21AB11 contained approximatively 500 HCV RNA copies/ml. Genotyping was not possible probably because of the low RNA copy number. HCV genotype 1b was detected in three patients, one patient sample was reactive for genotype 1 with no subtyping result.

Three patients had increasing HCV RNA concentrations during the first 2 to 4 months after infection, which decreased afterwards to an average value of 600,000 copies/ml serum 8 to 9 months later (Fig. 1A,C,D). Slowly increasing virus concentrations were observed in the fourth patient, who reached peak values only 7 months after infection (Fig. 1B).

One patient had normal liver enzyme levels over the whole observation period (Fig. 1A). Two patients (Fig. 1C,D) showed peaks of liver enzyme levels after 5 to 7 and 3 to 5 months, respectively, and decreasing levels afterwards. The high ALT levels of patient M.B. (Fig. 1B) were probably caused by Atovaquon (Wellvone®) therapy (Glaxo Wellcome, Hamburg, Germany). After its discontinuation liver enzyme levels returned to normal within a few weeks.

HCV seroconversion was observed in only two of the four infected patients, 2 and 9 months after Gammagard® application, respectively. In one patient (with 338 CD4+ cells/μl) (Fig. 1C) antibodies against the NS3 region of HCV were detectable 9 months after infection. Only one patient (with 139 CD4+ cells/μl at time of infection) had 5 months after infection serum antibodies against all three antigens used in the HCV Matrix assay (Fig. 1D). Antibodies against the HCV core region appeared 1 month after infection. Two months later, antibodies against the NS3-region of HCV and after 5 months immune reaction against the NS4 region was detectable. With appearance of antibodies the viral load decreased below the detection limit of RT-PCR, but HCV PCR was positive again 9 months after infection.

GBV-C/HGV RNA was detected by RT-PCR in the Gammagard® lot 93F21AB11. The results of GBV-C/HGV RNA and antibody determination in follow-up samples of the four patients are shown in Figure 1A–D. All the four patients were negative by the GBV-C/HGV-RT-PCR prior to therapy and until the end of the follow-up period. Anti-E2 antibody was detected in Gammagard® lot 93F21AB11 and in one patient before administration of immunoglobulin (Fig. 1A). Anti-E2 seroconversion was observed in one recipient (Fig. 1B) with index values close to the cut off, the other two patients remained anti-E2 seronegative until the end of the observation period (Fig. 1C,D).

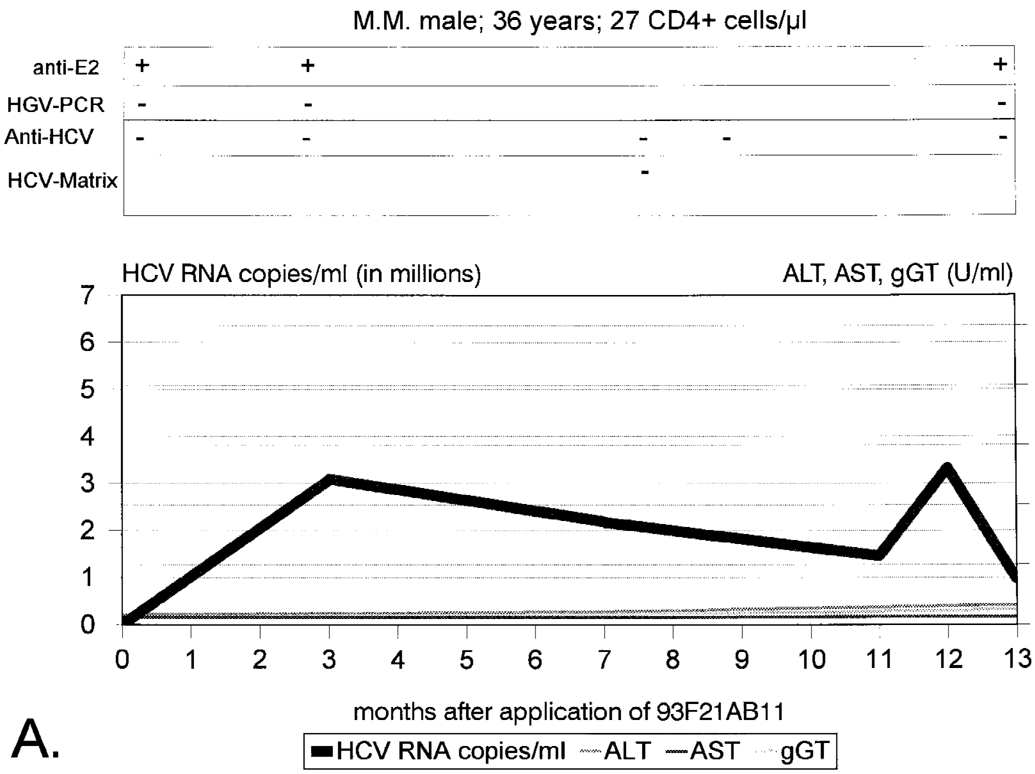
DISCUSSION

The present report documents acute HCV infection after administration of contaminated immunoglobulin in four HIV infected patients with severe hypogammaglobulinemia. Serum HCV RNA was present in all patients in the first sample tested, obtained 3–4 weeks after immunoglobulin administration. Most of the patients were highly viraemic during the acute phase of infection. While a significant decrease of HCV RNAemia was observed in two patients after the acute episode, all the individuals remained viraemic at the end of the follow-up.

Whereas viraemia was detectable in all the four patients 3–4 weeks after Gammagard® treatment, the immune response was highly variable. Two patients with relatively high CD4+ cell counts seroconverted 2 and 10 months after immunoglobulin donation. The other two individuals with advanced HIV infection failed to produce specific antibodies in response to acute HCV infection. In line with the findings of Naito et al. [1994] and Giuberti et al. [1994], in our patients, viral load decreased with seroconversion and in one patient HCV RNA was no longer detectable after seroconversion. A renewed increase in viral load was observed later. No patient with acute hepatitis C showed resolution during the follow-up period. It has been suggested that epitopes of neutralizing antibodies exists in the hypervariable region (HVR) of HCV and that the immune selection of escape variants during the course of chronic infection may be the mechanism of virus persistence [Kurosaki et al., 1993].

Elevated liver enzyme levels were observed only in the two patients who seroconverted. These findings suggest that liver injury may be related to an immune-mediated mechanism. Vento et al. [1996] observed that withdrawal of chemotherapy or glucocorticoids can give rise to massive liver cell necrosis in chronic HCV carriers suffering from malignancies. Reconstitution of a valid immune response in HCV-infected patients can be associated with life-threatening reactivation of liver disease.

All four patients became HCV infected. In contrast, GBV-C/HGV infection after donation of contaminated IVIG was not observed in any of the four cases. Anti-E2 antibody seroconversion in one patient cannot be considered as sufficient proof for GBV-C/HGV infection,



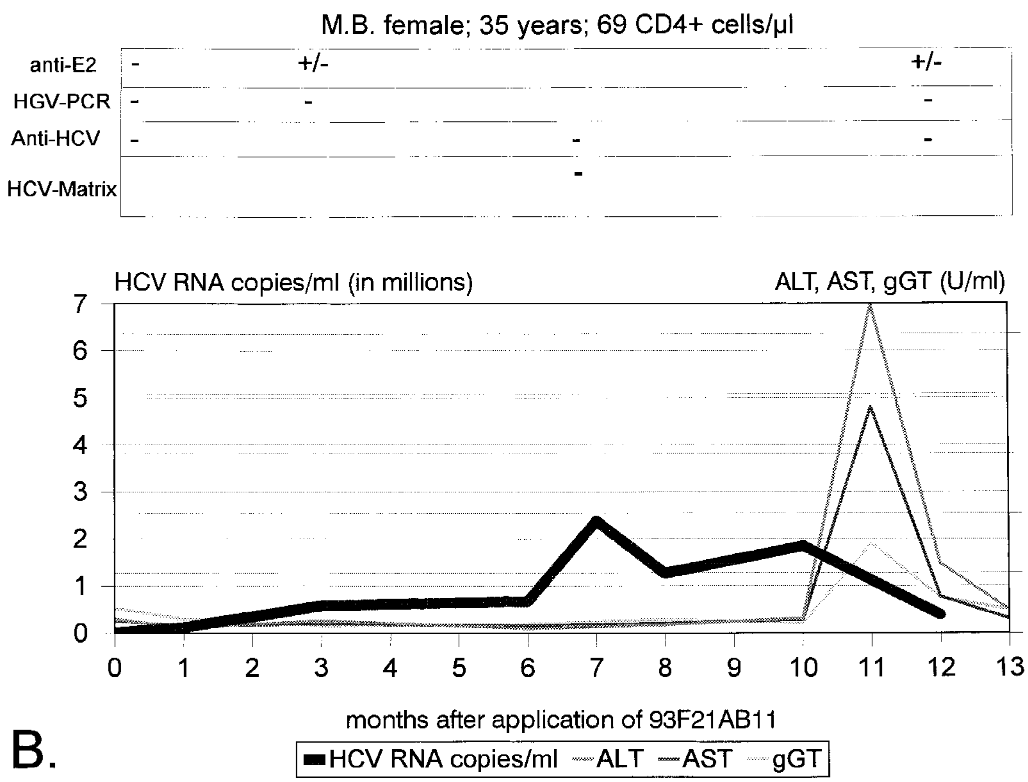


Fig. 1. **A–D**: Liver enzyme levels, GBV-C/HGV-RT-PCR results, anti-GBV-C/HGV, and HCV antibody response and HCV-RNA copies/ml in course of acute HCV infection in four HIV-positive patients.

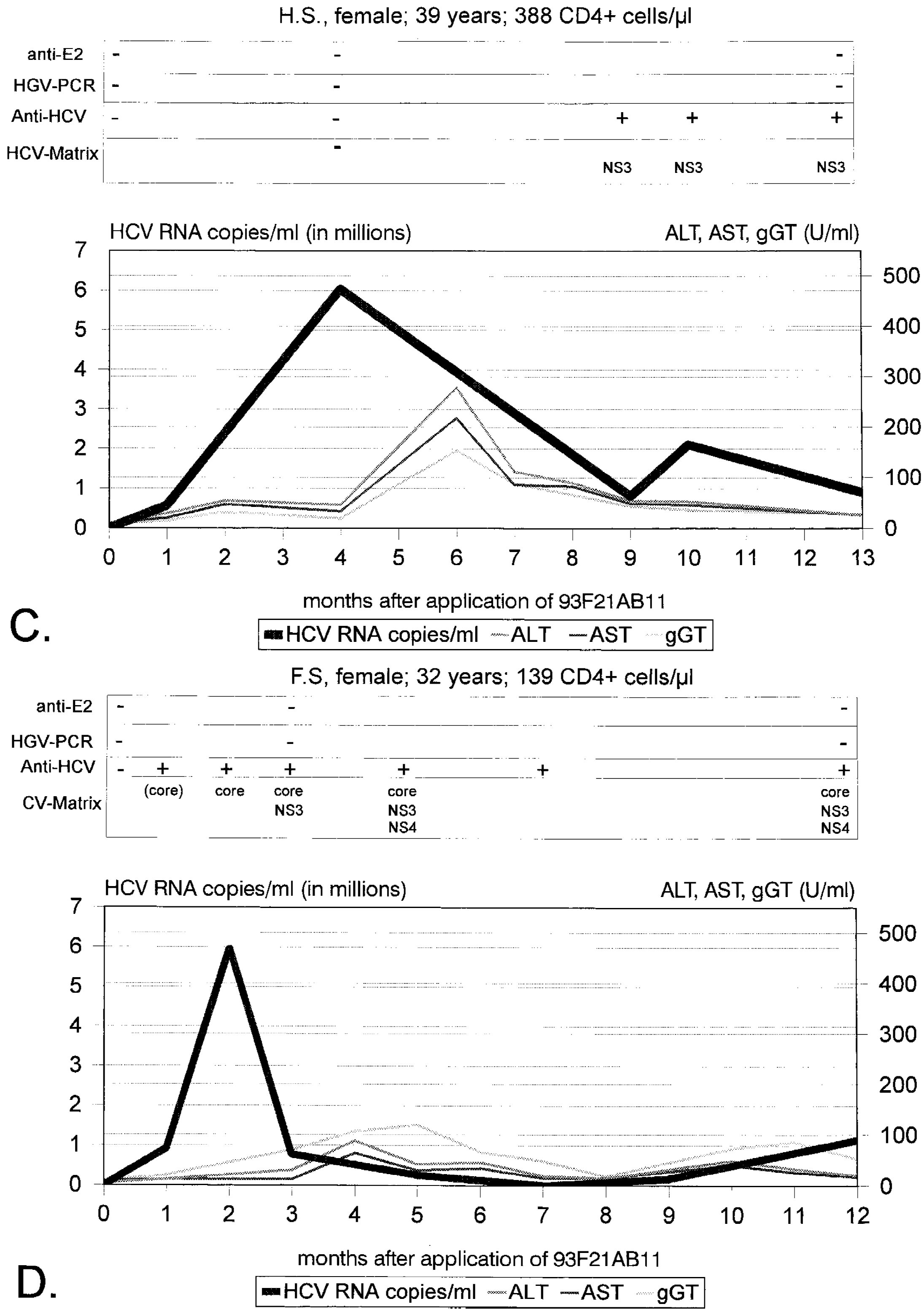


Fig.1. (*Continued*).

since in this particular case, the index values for GBV-C/HGV E2 antibody were close to the cut-off so that a nonspecific reactivity cannot be ruled out. There are several possible explanations for the failure of GBV-C/HGV transmission by IVIG. 1) The presence of anti-E2 antibody in the intravenous immunoglobulin may have had a protective effect in the recipients, i.e., neutralization of infectious virus. Furthermore, the presence

of neutralising antibodies could enhance the virus inactivation during in the manufacturing process. Antibody against the putative envelope is considered as a marker for past GBV-C/HGV infection. [Tacke et al., 1997]. The putative envelope proteins of GBV-C/HGV (E1, E2) are relatively conserved in comparison to those of HCV; there is no evidence for a hypervariable region to allow immune evasion [Jarvis et al., 1996]. 2) Detection of GBV-C/HGV RNA by RT-PCR does not necessarily correlate with the presence of infectious virus. 3) Since a high proportion of plasma pools and Gammagard® lots are contaminated with GBV-C/HGV, it could be supposed that the four recipients were immunized passively against GBV-C/HGV by previous and later repeated exposures to the infectious agent.

ACKNOWLEDGMENTS

We thank W. Preiser for critical reading the manuscript and M. Kortenbusch for her technical assistance.

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